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Sebaceous Gland Differentiation I. Separation, Morphology and Lipogenesis of Isolated Cells from the Mouse Preputial Gland Tumor

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Single cell suspensions have been prepared, by enzyme digestion, from the mouse preputial gland tumor and separated by flotation centrifugation into populations of different buoyant densities. These populations of cells have been shown by morphological, chemical and biochemical criteria to be in different stages of maturation. Some properties of the separated cells are described.

The sebaceous gland is a holocrine organ the cells of which become the final secretion. To form this secretion the basal sebaceous cell replicates and a daughter cell accumulates lipid during its development and undergoes a lethal type of differentiation. The morphology of this process, as seen under the light microscope, has long been recognized [1] and extensive electron microscopical studies [2,3] have been made, yet the basic biochemistry remains poorly understood. If sebaceous cells could be separated according to their state of development and in sufficient quantities to permit chemical and biochemical characterization, then progress would be greatly facilitated. As the sebaceous cell matures, lipids accumulate and the cell density should decrease. Hence, theoretically, it should be possible to separate such cells according to their state of development by use of appropriate density gradient centrifugation or a similar procedure based on density difference. The normal sebaceous gland of both man and animals is too small to be of practical use in these studies, but certain animals, especially rodents, possess specialized sebaceous glands which are large enough to be of use. Such glands are the preputial glands of the rat and mouse, the flank organ of the hamster and the ventral gland of the gerbil. The secretion of these glands plays a different physiological role from that of the normal sebaceous gland in that they are territorial markers, nevertheless they are holocrine glands in which the process of cell maturation is akin to that of all sebaceous glands. The mouse preputial gland is particularly amenable since it grows to a relatively large size (up to 150 mg for the pair of glands from a single animal); futhermore a transplantable tumor of the gland (ESR 586) is available.

In the study described here, the feasibility of this approach to cell separation has been explored with the preputial gland tumor as the selected tissue because it made possible the isolation of large quantities of sebaceous cells. For purposes of comparison some studies using normal preputial glands have also been made.

MATERIALS AND METHODS

Tissues

The original tumors were obtained from the Jackson Laboratory and were maintained by transplant into C57BL/6J mice. The mice were sacrificed at 3–4 weeks after transplant and the tumor was excised *in toto*. Normal preputial glands were obtained from mature nontumor bearing mice by dissection.

Tissue Disaggregation and Cell Harvesting

Hank's Ca and Mg-free basic salt solution was used for all experimental procedures.

Tumors. The tumor was carefully cut into small pieces and incubated with a volume of Hank's solution, containing Collagenase III (Worthington Biochemical Corp., 3 mg/ml), such that each ml of medium contained 50-100 mg tissue. The incubation was performed in a gyro-rotary shaker bath at 37°C with a shaker speed of 200 rpm. After incubating for 15 min, one-half volume of Hank's solution containing trypsin (Worthington Biochemical Corp., code TRL, 3 mg/ml) was added and the incubation continued for a further 30 min. At the end of this period one-half volume of Hank's solution containing soy bean trypsin inhibitor (Worthington Biochemical Corp., 2 mg/ml) and EDTA (3 mg/ml) was added and the incubation continued for a further 5 min. The digest was filtered through a 50 μm NITEX screen (Tekto Inc., HC-3-48) to remove undigested tissue and the filtrate centrifuged for 5 min at 450 ×*g* to harvest cells. A Sorvall RC-5 centrifuge was used throughout this work. This was operated at 20°C and fitted with an HB-4 swing-bucket rotor. Adapters, tubes and speeds were varied as required. To achieve the centrifugal force just specified we used a #367 adapter and 10 ml tapered Pyrex tubes with a speed of 2000 rpm.

Normal glands. The above procedure was modified slightly. The glands were carefully cut in half and 1 to 4 glands incubated with each ml of the collagenase solution. Incubation times were longer namely 45 min with collagenase and a further 30 min after adding trypsin. Harvesting was performed by centrifuging at a lower speed at 110 ×*g* (#402 adapter; 12 ml polycarbonate tubes; 1000 rpm) for 5 min.

Cell Separation

Tumor cells. The procedure is illustrated in the flow diagram (Fig 1). The cell pellet obtained by the preceding technique was washed twice by resuspending in Hank's solution, centrifuging and then discarding the supernatant. It was then suspended in 1 volume (usually 2 ml) of 40% w/v Ficoll 400 (Pharmacia Fine Chemicals). This solution was prepared by dissolving the Ficoll in Hank's solution and the density determined gravimetrically using a Gay-Lussac bottle. This was usually 1.135. A step-wise gradient was then created over this cell suspension by layering equal volumes of 35%, 30%, 25% and 20% Ficoll solutions (prepared by diluting the stock 40% solution with Hank's and determining the density of each solution gravimetrically) in sequence followed by a final column of Hank's solution. The gradient was then centrifuged for 30 min at 2800 ×*g* (#402 adapter; 12 ml polycarbonate tubes; 5000 rpm). Bands of cells collected at the interfaces of the gradient and were removed manually with Pasteur pipets starting with the top band and working downwards. The cells were harvested by diluting the Ficoll solutions with Hank's solution and then centrifuging at 450 ×*g* (#363 adapter; 15 ml COREX tube; 2000 rpm) for 5 min.

Normal gland cells. The above procedure was modified slightly. The washed pellet was suspended in 25% Ficoll. The gradient was created with equal volumes of 20%, 15%, 10%, 5% Ficoll and Hank's solution. The gradient was centrifuged for 30 min at 110 ×*g* (#402 adapter; 12 ml polycarbonate tubes; 1000 rpm). After dilution of the Ficoll suspensions, the cells were harvested by centrifuging for 5 min at 100 ×*g* (#363 adapter; 15 ml Corex tube; 1000 rpm).

Light Microscopy

Tumor tissue was processed for histology by standard procedures and sections stained with hematoxylin and eosin. Cells were collected on cellulose ester membranes (Millipore Corp., type SM, 5 μm pore, 25 mm diameter), fixed *in situ* and stained with a modified Papanicolaou method [4]. This enabled cells, which would not pellet due to low

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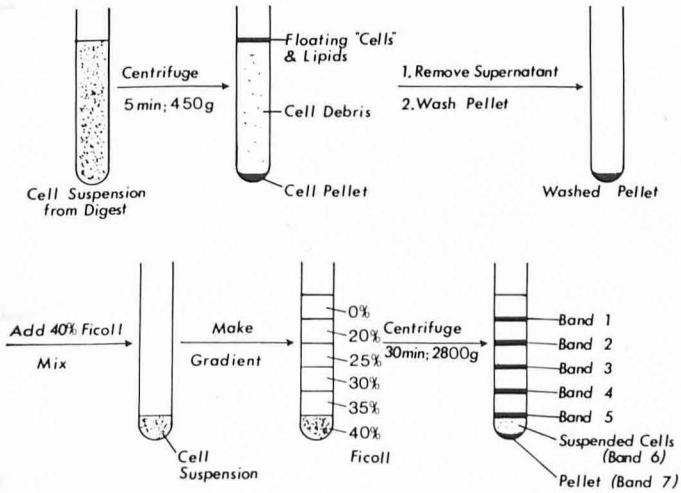


FIG 1. Flow diagram showing the method of separating tumor cells according to buoyant density.

buoyant density, to be collected for staining. Cell diameters were determined by measuring cells, stained for 30 min with toluidine blue (0.1%), using a micrometer eyepiece. Cell counts were performed by hemocytometer and viability determined by Trypan blue exclusion.

Electron Microscopy

Cells, collected on Millipore membranes (2×10^6 cells/cm^2), were partially fixed *in situ* by passing 4% glutaraldehyde through the filter. The membranes were then removed from their filter holders and fixed in 4% glutaraldehyde for 1 hr followed by fixation in 2% osmium tetroxide buffered to pH 7.4. They were then dehydrated rapidly in graded strengths of ethanol and embedded in Epon 812. Sections 1 μm thick and ultrathin sections were cut on a Reichert ultramicrotome, stained with uranyl acetate and then with lead citrate, and examined in a Siemans-Elmiskop 1A electron microscope.

Chemical Analyses

Protein was determined by the Lowry procedure [5]. Lipids were extracted from cells by the method of Vorbeck and Marinetti [6]. Total lipid was assayed by a micro modification of the method of Bloor [7]. Lipid class analysis was performed by the Downing procedure [8] which was modified in the following manner. The TLC plate was prewashed with chloroform-methanol (2:1) instead of ether. Two runs were made with cellular lipids. In the first run the plate was developed first in benzene and then in hexane, then charred and assayed as described by Downing [8]. In this run both polar lipids and free fatty acids remain at the origin. A second run was made in which the plate was developed first in benzene and then in Downing solvent No. 3 (hexane-ether-acetic acid). This second run separated the free fatty acids from the polar lipids and permitted appropriate correction of the first run. This modification was found necessary because in preliminary experiments, using the original Downing procedure, it was found that glyceryl ether diesters, triglycerides and alkyl acetate are not separated adequately for quantitation.

In the above procedures about 10^5 cells were used for the Lowry method and 10^6 cells used for each the Bloor and thin-layer procedures.

Enzyme Assays

Homogenates were prepared using a Dupour homogenizer (Kontes Glass Co., K-885250) followed by a brief sonication using a Branson Sonifier fitted with a microprobe. Glucose-6-phosphate dehydrogenase (EC 1.1.1.49), isocitrate dehydrogenase (EC 1.1.1.42), malic enzyme (EC 1.1.1.40), lactate dehydrogenase (EC 1.1.1.27), and malate dehydrogenase (EC 1.1.1.37) were assayed by the fluorometric methods of Halprin and Ohkawara [9] and Im [10]. Reaction rates were determined using an Aminco (Model 4-7390) fluorometer fitted with a strip chart recorder and the sets of filters described by Im and Hoopes [10]. Citrate cleavage enzyme (EC 4.1.3.8) was assayed by a fluorometric adaptation of the method of Srere [11], and acetyl-CoA carboxylase (EC 6.4.1.2) by a fluorometric adaptation of the spectrophotometric method of Numa [12]. About 5×10^6 cells were required for all the above assays.

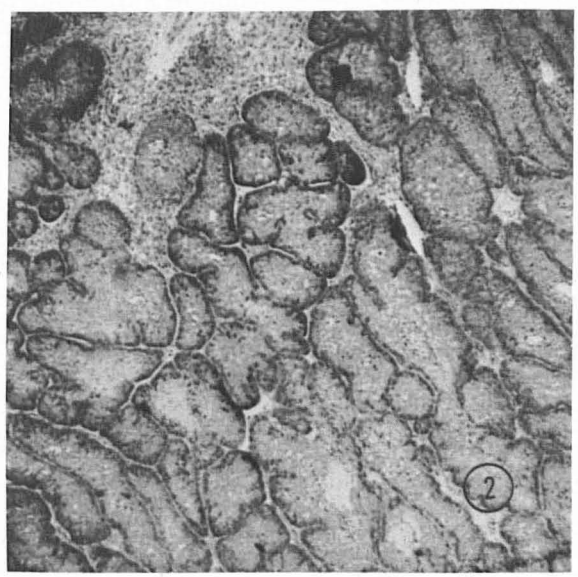
Precursor Incorporation Rates

Rates of incorporation of amino acids into protein were measured by incubating 1×10^6 cells with 1 μCi ^{3}H-protein hydrolysate (Schwarz-Mann) for 1 hr at 37°C. After incubation, the samples were filtered through Whatman GF/C filters (24 mm) and the filters washed with trichloroacetic acid as described by Everhart, Hauschka, and Prescott [13]. The filters were placed in scintillation vials containing Aquasol (New England Nuclear Co.) and counted in a Packard Tricarb liquid scintillation counter using the channels ratio method for efficiency-quench correction [14]. To measure lipid synthesis, 0.5×10^6 cells were incubated with 1 μCi ^{14}C-acetate (Amersham-Searle) for 3 hr at 37°C. The cells were saponified and the lipids extracted as described by Wheatley et al [15]. The extracted lipids were transferred to scintillation vials and counted in Aquasol.

RESULTS

Tumor Morphology

The mouse preputial gland tumor, ESR 586, is a discrete tumor encapsulated in mucoid-like material. It grows rapidly and usually exceeds 5 gm in mass at 5 weeks after transplant. The host animal always dies after successful transplant, usually at 6–7 weeks but never later than 14 weeks. The tumor is



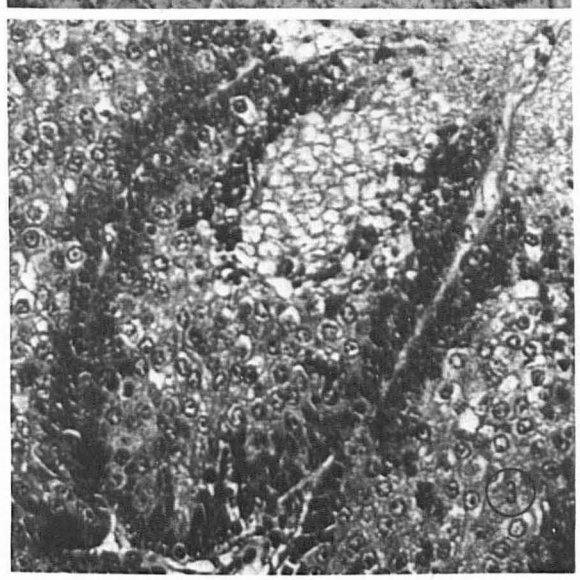


FIG 2. Low-power micrograph of the tumor showing the multi-acinar type of structure. × 50.

FIG 3. Higher power micrograph of an acinus in the tumor. The sebaceous type morphology is evident and is similar to that observed in the normal gland. × 250.

classified as an adenocarcinoma [16]. Histological examination shows a multi-acinar structure somewhat resembling the normal preputial gland but without the large ductular spaces seen in sections of the normal gland [17]. The low-power micrograph (Fig 2) shows the multi-acinar structure of the tumor while the higher power micrograph of a single acinus (Fig 3) shows the typical sebaceous appearance with the basal cells at the periphery and the more mature cells towards the center. Previously we have shown [18] that, as the tumor grows, the parenchyma increases while there is a decrease of the ductular areas. In the experiments described herein tumors of approximately the same age, i.e., in the same state of development, have been used.

Conditions for Tissue Disaggregation and Cell Harvesting

Since the sebaceous cells are unduly fragile, careful handling of cells and tissues is essential. An attempt to use the nonenzymatic method of Fidler [19] for tissue disaggregation was completely unsuccessful. For the enzymatic procedure, the tumor must be carefully cut into small pieces and drastic chopping avoided. Various enzymes, both singly and in combination were tried namely, Collagenase I and III, trypsin, papain and hyaluronidase. Best results were obtained by sequential treatment with collagenase and trypsin. Highest yields were obtained with relatively short digestion times and treatment with trypsin inhibitor and EDTA after enzyme digestion was found essential in order to prevent cell destruction and the formation of fibrin-like clots which clumped cells together. A shaker speed of 200 rpm in a gyro-rotary shaker-bath gave best results in that the yield fell at lower speeds while higher speeds caused cell destruction. A reciprocating shaker was also used satisfactorily but must be operated at a slower speed (100 rpm). The above treatment resulted in a virtually monodisperse cell suspension. Yields were good: an average of 2×10^8 viable cells/gm tissue with viability from 87 to 99% (average 93%). This compares favorably with high yield methods for other tissues [20,21]. The cells from the tumor range in size from 6.5 to 19 μm in diameter with population distribution as shown in Fig 4.

The normal glands require even more careful treatment and must not be squeezed as they are removed from the animal. Since the gland is encased in a keratin capsule [18], little disaggregation results if the gland is incubated whole and it is necessary to expose the inner surface of the gland to the digestive enzymes. In the experiments described here the gland was cut in half vertically and yields of 3×10^7 viable cells/gm tissue obtained with viability from 60% to 80% (average 72%)—significantly lower than those obtained from the tumor tissues. The cells range from 6.5 to 32 μm in diameter with population distribution shown in Fig 4.

The cells in the suspensions obtained from these digestion procedures can then be harvested. This is best accomplished by centrifuging at low speeds. The tumor cells are relatively robust and can be harvested and washed by centrifuging for 5 min at 450 $\times g$ without significant loss. After the initial centrifuging of the suspension, lipid droplets and cellular debris (see below) collect at the top, the supernatant contains cellular debris only, while the cells collect as a pellet at the bottom of the tube. Because the normal cells are more fragile, harvesting must be performed by centrifuging for 5 min at 110 $\times g$ and even at this speed some losses occurred. When the normal cell suspension is first centrifuged, large mature cells and lipid droplets collect at the top, the supernatant contains much cellular debris and some large cells, while the rest of the cells collect at the bottom of the tube. The cells are distributed roughly as follows; 11% at the top, 25% in the supernatant and 64% in the pellet.

Conditions for Cell Separation

Sedimentation under unit g, as described by Shall [22], was first tried as a method of cell separation but without success; the method was slow and no clear-cut separations were obtained. All attempts to separate cells by layering the suspension on the top of a sucrose or Ficoll gradient resulted, on centrifuging, in almost total destruction of both normal and tumor cells. Satisfactory separation could only be accomplished by flotation upwards through the gradient. Linear gradients were first tried but failed to produce discrete bands of cells, while, in the stepwise gradient, Ficoll was found to be superior to sucrose. Sebaceous cell maturation is a continuous process and, while Tosti [23] has described 5 morphologic stages, transition from one stage to the next is gradual. In the procedure developed by us, the selection of the steps of the gradient was made empirically, hence each band of cells would not be expected to be absolutely distinct with regard to cell type but each would represent a narrow range of the total cell population. Some earlier experiments with tumor cells were performed with a slightly lower density gradient using 33%, 29%, 25%, 21% and 16.7% Ficoll solutions but this was later discarded. In certain experiments, however, where we wanted better resolution of the lower density tumor cells, we added additional layers of 15% and 10% Ficoll to the normal range of densities. In all experiments the buoyant density of a given band of cells was determined by calculating the mean density of the Ficoll layers above and below the band. Cells which pelleted in 40% Ficoll were found to have an average density of 1.15.

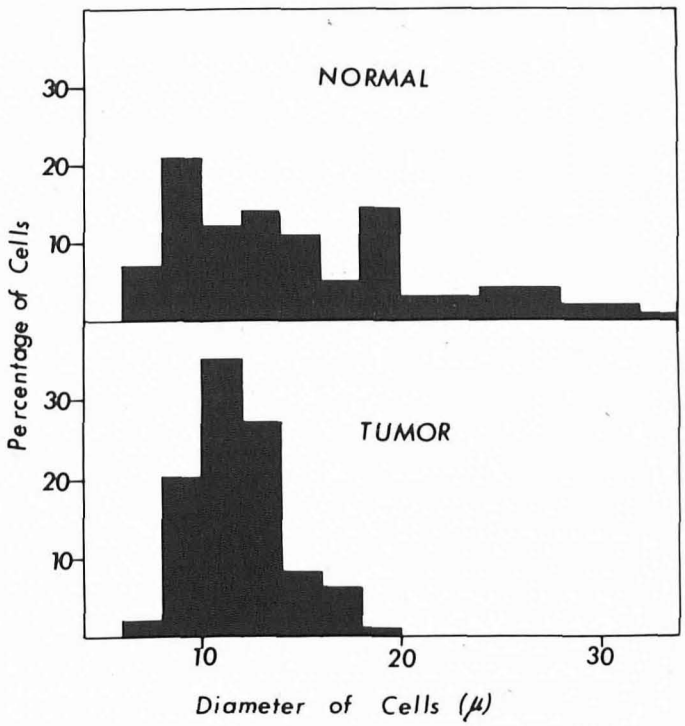


FIG 4. Histogram showing the distribution of the normal gland and tumor cells according to cell diameter.

Morphology of Separated Tumor Cells

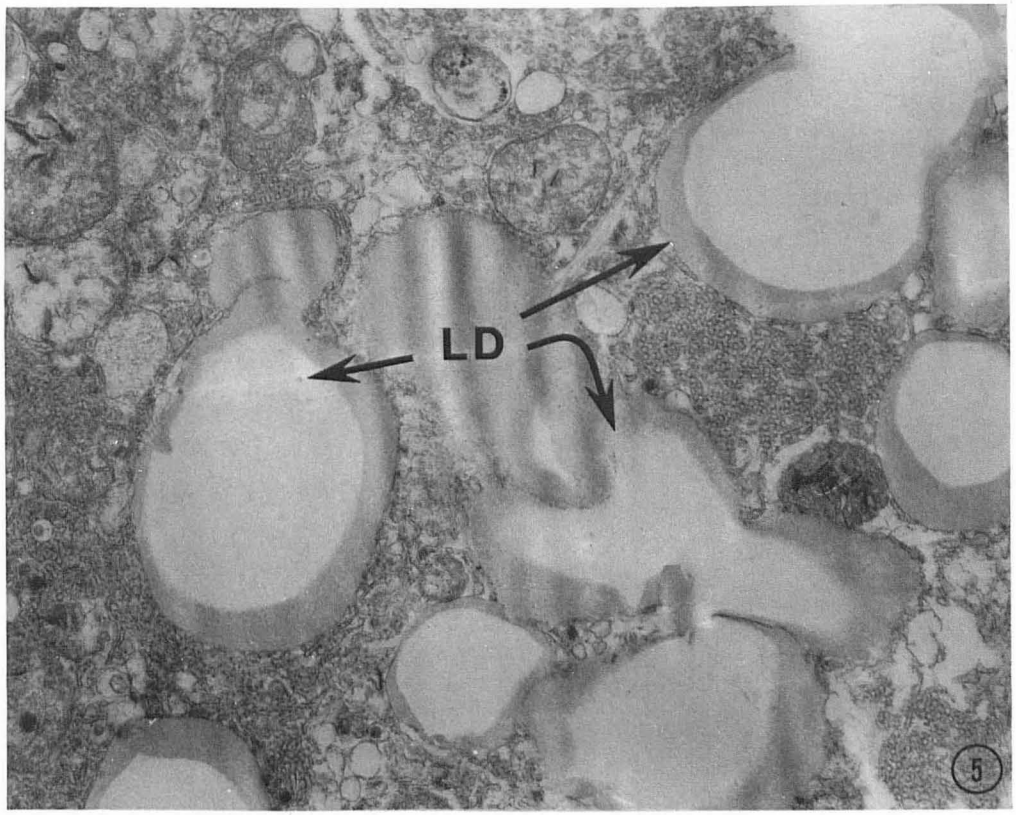
The cells in each separated band were examined by both light and electron microscopy. For numbering of bands etc., refer to Fig 1.

Light microscopy. Examination of tumor cells by the Millipore procedure showed the following general features.

Band 1. The cells of this band had the lowest buoyant density and were large with many cytoplasmic vacuoles.

Bands 2 to 6. The cells in these bands showed decreasing cytoplasmic area and the presence of less vacuoles as the buoyant density decreased. The cells were increasingly more densely stained, the nucleus more basophilic and the cell diameter smaller as the density increased.

Band 7. The cells in the pellet were small and densely basophilic. The nucleus was large and there was relatively little cytoplasm, only occasionally were vacuoles seen in the cyto-



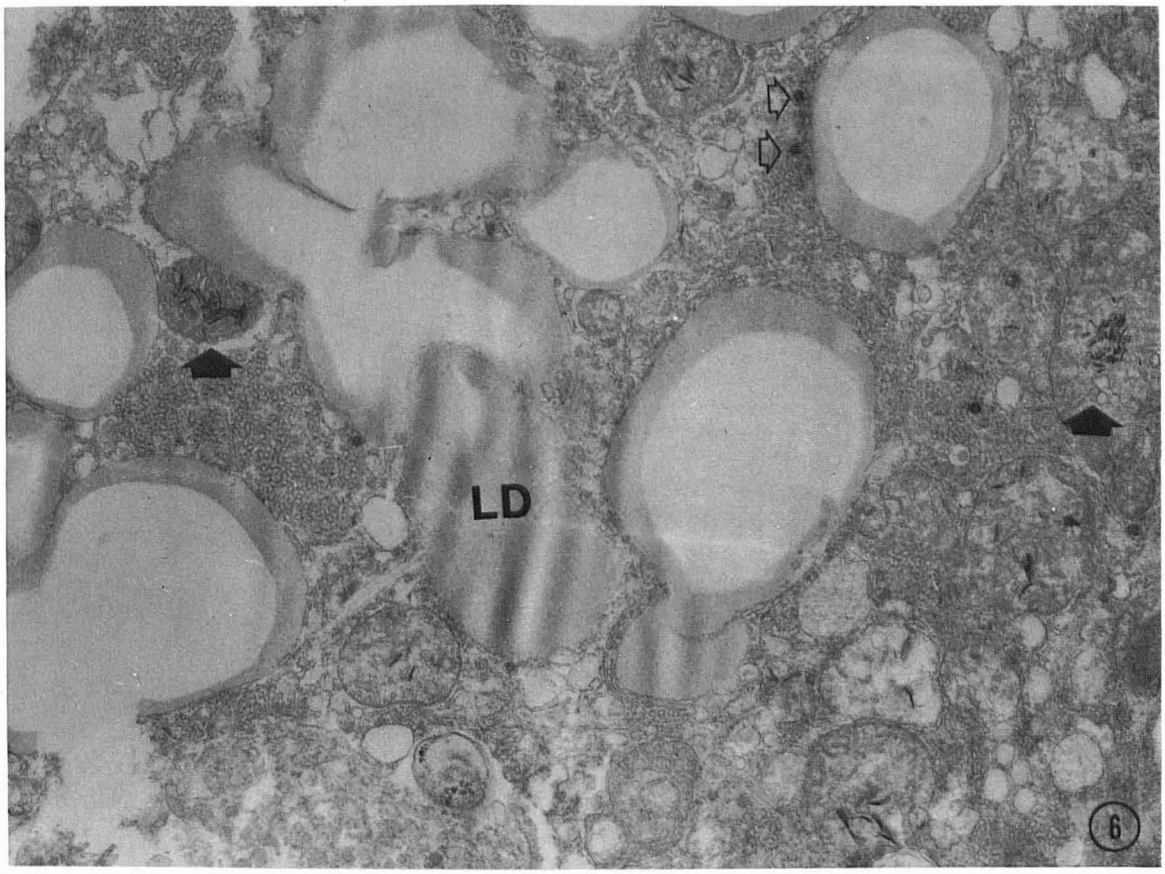
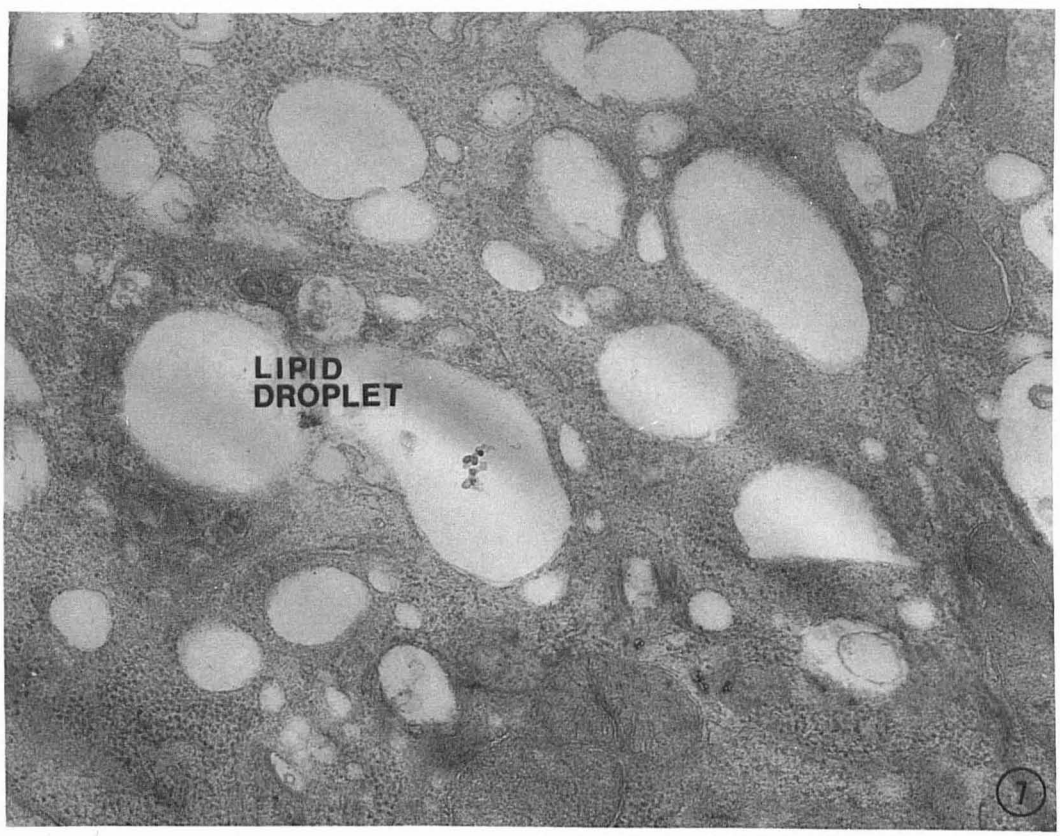


FIG 5. Micrograph depicts the large lipid droplets (*LD, arrows*) in the tumor cells from band 1. × 18,700.

FIG 6. Many of the mitochondria in the cells of band 1 contain several osmiophilic spicule-like structures (*black arrow*). The *clear arrows* point to viral particles. × 18,200.



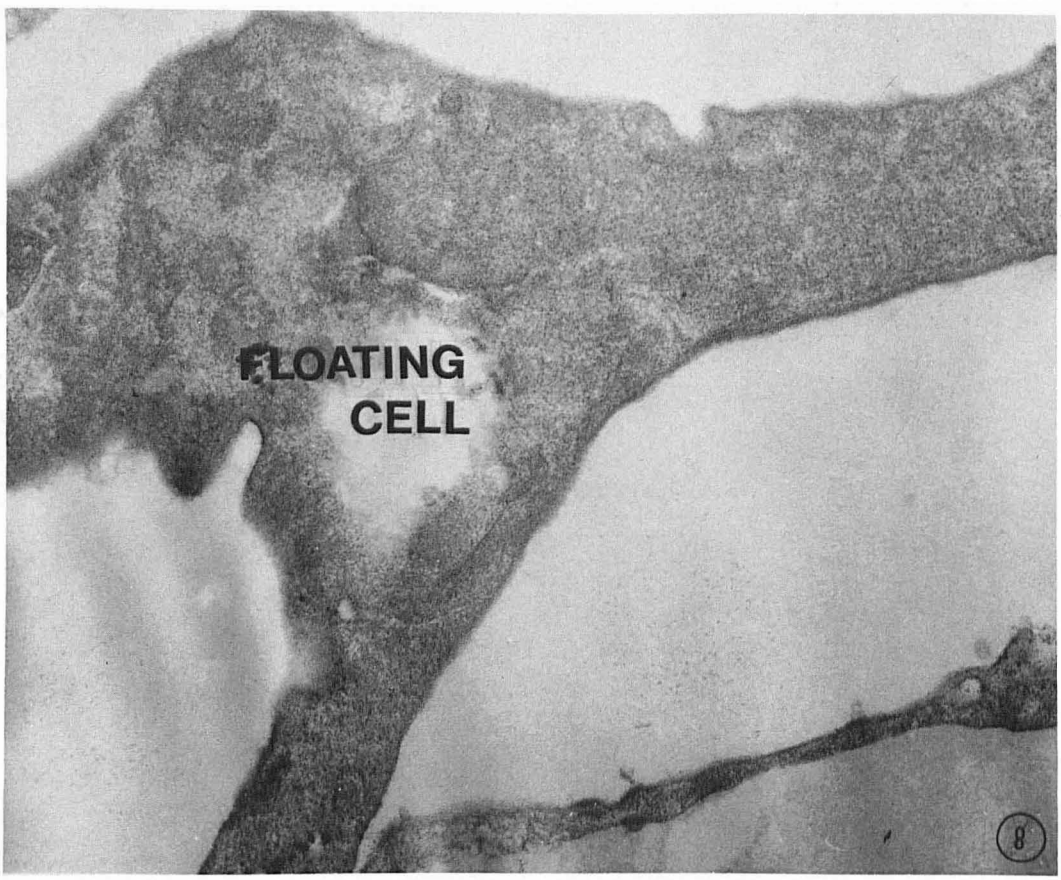


FIG 7. In the cells of band 5, there are more small lipid droplets than the large droplets characteristic of the cells of bands 1–4. × 36,000.
FIG 8. Micrograph depicts 2 floating "cells." These cells are narrow in profile, amorphous with few organelles. × 43,000.

plasm. One or 2 larger less densely stained cells were also seen. These large cells were subsequently shown to be nonviable.

Floating "cells". When the floating band of lipid droplets and debris was examined by this technique, no recognizable cells were seen but a number of anuclear squama-like "cells" were observed.

Electron microscopy. Examination of the separated tumor cells by electron microscopy showed the following features.

Band 1. The cells of this band contained large lipid droplets several of which appear to be coalescing (Fig 5). The cytoplasm of these cells also possess numerous Golgi vesicles and rough surfaced endoplasmic reticulum. The latter occasionally have in their cisternae 1 or 2 viral particles. Many mitochondria contain several osmiophilic spicule-like structures between the cristae (Fig 6).

Bands 2 and 3. In these 2 bands, the cells possess both large and small droplets. The rough endoplasmic reticulum contains a few viral particles and the numerous mitochondria possess the osmiophilic spicule-like structures.

Band 4. In the cells of this band one sees an approximately equal amount of both large and small lipid droplets. The Golgi apparatus is extensive with several vesicles. Viral particles are sparse and the mitochondria now do not possess the spicule-like structures.

Band 5. It is in some of the cells of this band that one can observe a flattening of the height of the cell as well as an increase in the nucleus:cytoplasm ratio. In addition, there appears to be an increase in small lipid droplets (Fig 7).

Bands 6 and 7. Most of the cells in these 2 bands are flattened with an increased nucleus:cytoplasm ratio when compared with the previous bands. In band 7, there are a few lipid droplets in the cells and viral particles are never present.

Floating "Cells". These "cells" are all narrow in profile, are amorphous with little or no organelles and have thickened plasma membranes (Fig 8). Occasionally, 1 or 2 lipid droplets can be observed with a few vesicles in some "cells."

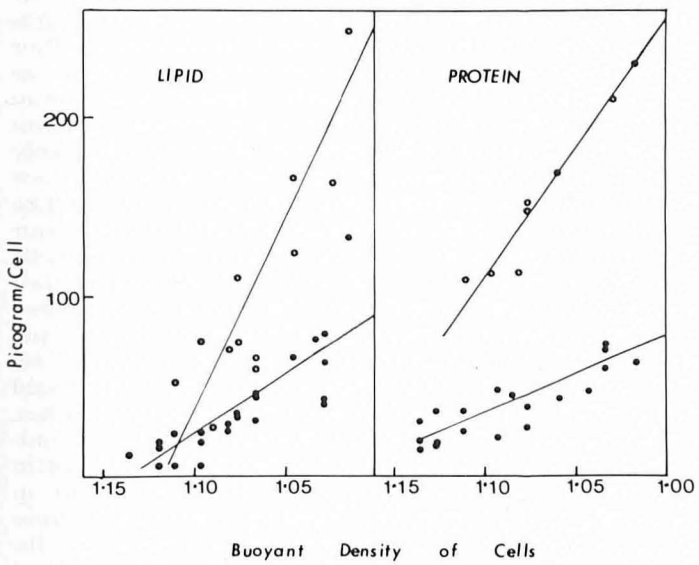


FIG 9. The lipid and protein content of normal gland (O) and tumor cells (●) of different buoyant densities.

Biochemical Features of Separated Tumor Cells

The lipid content of both normal and tumor cells of different buoyant densities is shown in Fig 9. There is a marked increase in lipids for both normal and tumor cells as the buoyant density decreases. At the same time there are changes in the composition of the cellular lipids. This is shown in the Table which represents data from a single experiment selected from several similar experiments each showing the same general trend. There is an increase, both absolute and relative, in waxes and steryl esters while free sterols and polar lipids decrease in concentration but the absolute amounts show some variations without any obvious correlation. The lipid composition of mixed normal cells is shown for comparison. The tumor cell lipids never reach the high concentration of waxes found in the normal cells (maximum 9.9% compared with 31.1%), while the alkyl acetates of normal cells are never found in the tumor cells. Squalene, on the other hand, is present in the tumor cells but not in normal cells.

The protein content of both normal and tumor cells is also shown in Fig 9. Here again there is a marked increase in protein for both normal and tumor cells as the buoyant density de-

Lipid composition and lipid content of separated tumor cells

Component	Tumor cells							Mixed normal cells Composition (%)
	Band number and buoyant density of cells							
	7	6	5	4	3	2	1	
	1.150	1.135	1.127	1.097	1.081	1.053	1.019	
	Composition (%) and cell content (picograms)[a]							
Polar lipids	35.2	20.2	16.5	29.9	26.2	19.6	10.4	19.4
	4.5[a]	2.5	3.0	7.1	9.7	6.4	7.9	
Free fatty acids	8.4	5.5	5.6	5.6	7.1	2.8	1.0	3.0
	1.1	0.7	1.0	1.4	2.6	0.9	0.8	
Free sterols	11.1	11.4	8.9	7.4	6.4	5.4	3.7	6.1
	1.4	1.4	2.0	1.8	2.4	1.8	2.8	
Triglycerides	24.1	37.2	35.8	29.1	21.4	29.6	27.2	6.1
	3.1	9.6	6.7	7.0	7.9	9.7	20.9	
Glyceryl ether diesters and neutral plasmalogens	12.6	9.7	13.3	13.9	15.3	12.6	9.2	7.1
	1.6	1.2	1.8	3.3	5.7	4.1	7.0	
Alkyl acetates	0	0	0	0	0	0	0	1.2
	0	0	0	0	0	0	0	
Waxes	0.5	1.6	1.6	1.6	2.8	3.9	9.9	31.1
	0.1	0.1	0.3	0.4	1.0	1.3	7.6	
Steryl esters	5.0	10.6	13.5	8.2	14.2	21.5	33.1	19.1
	0.6	1.3	1.9	2.0	5.2	7.2	25.4	
Squalene	0.9	1.4	2.0	1.3	2.6	2.4	3.9	0.0
	0.1	0.2	0.3	0.3	1.0	0.9	3.0	

[a] Lower figure gives the cell content in picograms.

creases. The increase is of about the same magnitude as that for lipids.

The relative rates of synthesis by the tumor cells of protein and lipid, as measured by the incorporation of the appropriate precursor, is shown in Fig 10. Protein synthesis shows a steady decrease as the cells decrease in buoyant density while lipid synthesis increases. The activities of the enzymes (Fig 11) used to generate NADPH for lipid synthesis (glucose-6-phosphate dehydrogenase, isocitrate dehydrogenase, malic enzyme and malate dehydrogenase) all show progressive increase as the buoyant density decreases but a similar change in the more

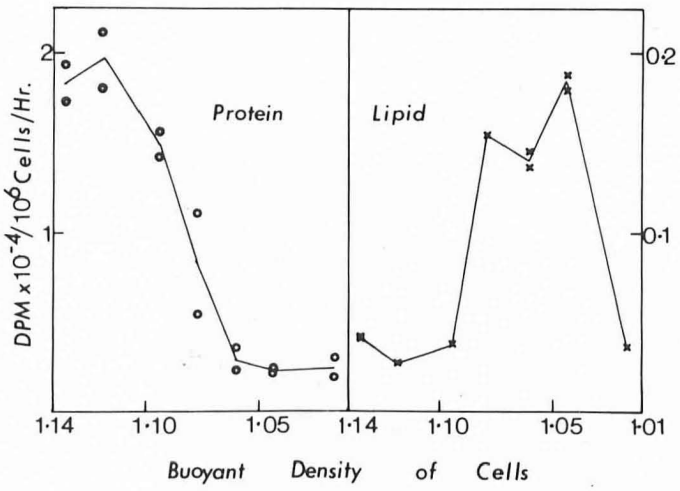


FIG 10. Relative rates of synthesis of protein and lipid by tumor cells of different buoyant densities.

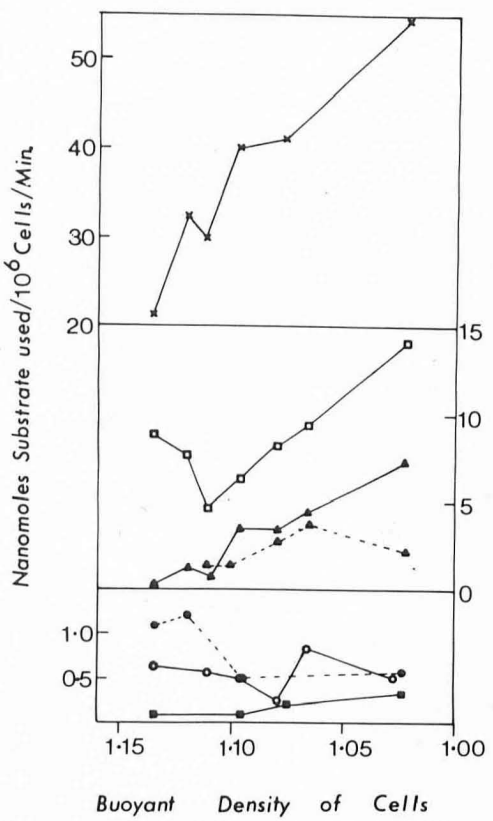


FIG 11. The variations of enzyme activities in tumor cells of different buoyant densities. The enzymes determined were malic enzyme (■), citrate cleavage enzyme (○), acetyl-CoA carboxylase (●), isocitrate dehydrogenase (△), glucose-6-phosphate dehydrogenase (▲), lactate dehydrogenase (□), and malate dehydrogenase (×).

characteristic enzymes of fatty acid synthesis (citrate cleavage enzyme and acetyl-CoA carboxylase) has not been demonstrated.

While the studies on normal cells have not been extensive, nevertheless certain differences between these and the tumor cells are already apparent. These differences have been summarized below:

a. Tumor cells never exceed 20 μm in diameter whereas 33% of normal cells fall in this range (Fig 4).

b. Tumor cells contain less lipid and protein than normal cells of the same buoyant density (Fig 9).

c. The wax content of tumor cells is much lower than that of normal cells (Table).

DISCUSSION

Single cell suspensions have been obtained, by enzyme digestion, from the mouse preputial gland tumor and from the normal preputial gland. The morphological features of these cells are those of a mixture of sebaceous cells in various stages of maturation. The densities of these cells are somewhat higher than anticipated. It was calculated that, if the increase in size of the sebaceous cell was due entirely to lipid accumulating in the vacuoles, then when the cell reached a diameter of 10 μm its density would be 0.99. The density would then progressively decrease, approaching that of sebum (0.910), as the cell grew larger. Hence all cells of a diameter of 10 μm or larger would be expected to float in Hank's solution (density, 1.005). In our experiments we found that some 80% of the isolated tumor cells had diameters greater than 10 μm (Fig 4) yet no nucleated floating cells were observed. This was fortuitous since it enabled complete separation of tumor cells to be performed by a simple density gradient centrifugation technique. Cells separated by this technique show the morphological differences that would be expected of sebaceous cells at different stages of maturation. In addition, cells which exhibit, morphologically, more advanced stages of maturation show corresponding increases in lipid content and rate of lipogenesis as well as an increase in most, but not all, of the enzymes involved in lipogenesis. These increases paralleled the decrease in buoyant density of the cells. We present this as evidence that the cells of the preputial gland tumor are undergoing a process of sebaceous-like differentiation and that the technique described is able to separate these cells into populations at different stages of this differentiation.

Two interesting observations are reported concerning these sebaceous type cells. First during maturation there is apparently an increase in protein content of the cells as well as an increase in lipid content. This was observed in both normal and tumor cells and is therefore not a peculiarity of the tumor cells. Unlike the preputial gland of the rat, which is a "dicrine" organ [24] and secretes a mixture of lipids and proteins, the mouse gland secretes only lipids (Wheatley, unpublished observations). Hence this does not represent a distinctive feature of the preputial gland, but could apply to other types of sebaceous glands. The increase in protein is so large that it is difficult to account for its presence and the possibility that the lipid vacuoles of these cells contain a lipid-protein complex rather than a pure lipid must be considered. In preliminary experiments we have detected, in homogenates from both the tumor and the normal gland, the presence of low molecular weight lipoproteins by gel electrophoresis. These are being further investigated. The protein content of the cells continues to increase (Fig 9) even when the rate of synthesis, as measured by isotope incorporation, has dropped well below the maximum (Fig 10). The amount of protein in the cell at a given time is determined both by the rate of synthesis and the time for which this rate is maintained. In cells of buoyant density of 1.05 and lower, the rate of protein synthesis is still some 10% of the maximum and these cells probably maintain this rate for longer periods so that significant increase in protein content still occurs. The data does not permit comparison of absolute rates of protein and

lipid synthesis, nevertheless, it suggests that in the early stages of maturation the cell is accumulating protein more rapidly than lipid. It is possible, therefore, that at the onset of differentiation the cell density does not decrease but actually increases. Our attempts to separate undifferentiated from early differentiating cells have been unsuccessful presumably because, for the above reasons, the density difference is too small.

The second observation refers to the changes in lipid composition of the tumor cells as they decrease in buoyant density. Similar changes have been observed in preliminary experiments with normal cells so this is not a feature unique to the tumor cell. Studies of the skin surface lipids of man by Downing and Strauss [25,26] have indicated that changes may occur in the lipid composition of the sebaceous cell as it matures and this is also implied in the studies on isolated human sebaceous glands by Summerly [27], but direct evidence has been lacking. These observations are of interest since they suggest that sequential gene activation may be occurring during the maturation of the sebaceous cell and that the term cytodifferentiation may, justifiably, be used to describe this process.

Even though less extensive studies have been made with normal gland cells, nevertheless the evidence so far available shows distinct differences from the tumor cells. Hence, while the tumor enables large numbers of cells to be easily collected for use in biochemical investigations, the data from such studies will need to be interpreted with caution until it can be confirmed on normal cells. Further studies with normal cells will define more clearly the uses and limitations of the tumor cells in the study of sebaceous cell differentiation. The cells obtained from both the tumor and the normal gland by these procedures are viable cells which are suitable for chemical and biochemical assays of relatively short duration. They are not intended for long-term incubation studies for which the cloned cell lines previously described by us [28] would be more suitable. Their advantage over the cultured cells is that they represent cells as they exist in the parent tissue and have not been exposed to an artificial environment as occurs with cells maintained in culture.

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Announcement

The Fourth Conference on Cutaneous Toxicity sponsored by the American Medical Association and the Society of Toxicology will be held at the Mayflower Hotel, Washington, D.C., May 9–11th, 1979. This continuing medical educational activity is acceptable for 12 credit hours in Category No. 2 for the Physician's Recognition Award of the American Medical Association. Registration fee $150 ($120 for AMA and SOT members; $85 for residents and retired physicians). For further information contact Dr. Joseph B. Jerome, American Medical Association, 535 North Dearborn Street, Chicago, Illinois 60601.